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Animal Model

Deletion of Decay-Accelerating Factor (CD55) Exacerbates Autoimmune Disease Development in MRL/lpr Mice

Takashi Miwa,* Michael A. Maldonado,† Lin Zhou,* Xiujun Sun,* Hong Yuan Luo,† Dewei Cai,‡ Victoria P. Werth,‡ Michael P. Madaio,§ Robert A. Eisenberg,† and Wen-Chao Song*

From the Department of Pharmacology, Center for Experimental Therapeutics; the Department of Medicine,† Division of Rheumatology; the Department of Dermatology;‡ and the Department of Medicine,§ Renal-Electrolyte and Hypertension Division, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania*

Decay-accelerating factor (DAF, CD55) is a glycosylphosphatidylinositol-anchored membrane protein that restricts complement activation on autologous cells. It is also a ligand for CD97, an activation-associated lymphocyte antigen with seven transmembrane domains. It is widely expressed on cells of both the hematopoietic and nonhematopoietic lineages. Although deficiency of DAF on human erythrocytes is associated with the hemolytic anemia syndrome paroxysmal nocturnal hemoglobinuria, the *in vivo* biology of DAF is still poorly understood. We addressed the *in vivo* function of DAF in a knockout mouse model and describe here that deletion of DAF exacerbates autoimmune disease development in MRL/lpr mice, a model for human systemic lupus erythematosus. Compared to DAF-sufficient littermate controls, DAF-deficient female MRL/lpr mice developed exacerbated lymphadenopathy and splenomegaly, higher serum anti-chromatin autoantibody levels, and aggravated dermatitis. Consistent with the phenotype of aggravated dermatitis in DAF-deficient mice, Northern and Western blots and immunofluorescence studies showed DAF to be expressed abundantly in the mouse skin, suggesting that it may play a particularly important role in this tissue. Histology and immunostaining demonstrated inflammatory infiltrate and focal C3 deposition in early skin lesions, mostly along the dermal-epidermal junction. These results reveal a protective function of DAF in the development of a systemic autoimmune syndrome and suggest that dysfunction or down-regulation of DAF may contribute to autoimmune disease pathogenesis and manifestation.
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Decay-accelerating factor (DAF, CD55) is a glycosylphosphatidylinositol-anchored membrane protein that inhibits C3 activation in both the classical and the alternative pathways.[1] Recently, DAF has also been identified as a ligand for an activation-associated lymphocyte antigen CD97,[2,3] suggesting it may have other noncomplement-related function(s). DAF is widely expressed on cells both within and outside the vascular space such as blood cells, endothelial cells, and may kinds of epithelial and stroma cells.[4,5] Although dysfunction of DAF on human blood cells contributes to erythrocyte sensitivity to complement lysis and increases the risk of thrombotic events in human paroxysmal nocturnal hemoglobinuria patients,[6,7] so far relatively little is known about the *in vivo* biology of DAF in other tissues and cells or disease processes. Based on the fact that DAF is one of the central membrane-bound complement regulators with a ubiquitous tissue distribution pattern, we hypothesized that DAF may be protective in settings of pathogenic antibody-mediated systemic autoimmune diseases in which complement activation is thought to contribute significantly to end organ damage.[8,9]

Complement plays a paradoxical role in the development and manifestation of systemic autoimmune diseases. Although deficiency of early complement components such as C1q and C4 predisposes individuals to the development of systemic lupus erythematosus (SLE), consumption of serum complement proteins and complement deposition in tissues are hallmarks of disease activity in SLE.[9] This apparent paradox reflects, on the one

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T. M. and M. A. M. contributed equally to this work.

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Address reprint requests to Wen-Chao Song, Room 1351 BRBII-III, 421 Curie Blvd., Philadelphia, PA 19104. E-mail: song@spirit.gcrc.upenn.edu.

hand, the facilitating role of complement (particularly its early components) in the disposal of apoptotic cell-derived autoantigens and circulating immune complexes and maintenance of tolerance[10–12] and, on the other hand, the inflammatory and cytolytic injury mediated by complement once SLE has developed.[8,9]

The role complement plays in the effector phase of systemic autoimmunity has been addressed recently in a murine model of SLE, the MRL/MpJ-*Tnfrsf6*^{lpr} (MRL/lpr) mice.[13,14] MRL/lpr mice have the *fas*^{lpr} mutation, which inhibits the expression of Fas, a cell surface apoptosis receptor in the TNF-R gene family.[15] In addition, the MRL background is autoimmune. MRL/lpr mice spontaneously develop an autoimmune syndrome characterized by elevated levels of Ig, multiple autoantibodies, nephritis, and vasculitis, in association with massive lymphoproliferation.[16] In transgenic MRL/lpr mice, inactivation of the complement factor B gene (Bf) was shown to be moderately protective,[13] whereas deficiency of the complement component C3 failed to attenuate either the progression or severity of autoimmune diseases.[14] Because Bf is located within the MHC class III locus, the possibility that H-2 differences might have also contributed to disease attenuation observed in MRL/lpr-Bf knockout (KO) mice has not been excluded.[13] Thus, the involvement of complement in the effector phase of the MRL/lpr model of systemic autoimmune disease is still not clearly defined.

One reason for the apparent lack of a detectable influence of C3 deficiency on disease development in MRL/lpr mice may be that the beneficial and detrimental effect of C3 has canceled each other.[14] Factors that can preferentially affect one or the other arm of complement activity may tip the balance toward a particular direction, depending on the circumstances. Such factors may include membrane-bound inhibitors of complement activation such as DAF. In the current study, we have addressed this hypothesis by investigating the effect of DAF deficiency on autoimmune disease development in MRL/lpr mice. Two homologous DAF genes, arranged in tandem on chromosome 1, have been identified in the mouse.[17] One DAF gene, referred to as the GPI-DAF (or Daf-1) gene, is predicted to encode a GPI-anchored protein similar to human DAF.[18–20] The second mouse DAF gene, referred to as the TM-DAF (or Daf-2) gene, is predicted to encode a transmembrane form of DAF.[18–20] However, minor alternatively spliced mRNA species from the TM-DAF gene, predicted to make GPI-anchored DAF, have also been detected.[21] The tissue expression patterns of the two mouse DAF genes are rather distinct. The GPI-DAF gene is expressed ubiquitously in all mouse tissues, whereas the TM-DAF gene is expressed only in the testis[5,18,19,22] and possibly in trace amounts in the spleen.[18,22] In this study, we have crossed a GPI-DAF gene KO mouse, previously generated in our laboratory,[23] with the MRL/lpr mouse, and compared autoimmune disease development in GPI-DAF gene-sufficient and gene-deficient littermate controls. Our results reveal a significant protective role for DAF in this model of systemic autoimmune disease.

Materials and Methods

MRL/lpr-DAFKO Mice

A KO mouse deficient in the GPI DAF gene (DAFKO) was generated as previously described.[23] DAFKO mice were crossed with MRL/lprIgh^{b} mice (MRL/lpr mice congenic for the Ig heavy chain b allele, maintained in our animal colony) for a total of nine generations. The DAFKO locus was identified by Southern blot analysis of tail DNA.[23] Four-time backcrossed or nine-time backcrossed DAF heterozygous MRL/lprIgh^{b} mice were used to derive wild-type (WT) and DAFKO MRL/lprIgh^{b} mice as littermates. Screening of mice was performed by a combination of Southern blot analysis of tail DNA[23] and fluorescence-activated cell sorting (FACS) analysis of erythrocyte DAF expression.[5] Mice were monitored for autoimmune disease until they were 5 months old, at which time they were sacrificed for pathological evaluation. All mice were kept in a specific pathogen-free barrier facility.

Determination of Serum Total IgG, IgM, and Autoantibody Levels

Mice were bled monthly starting at 3 months. Serum samples were stored at −80°C until analysis. Total serum IgG, IgM, IgG2a, anti-single-strand DNA (ssDNA), anti-double-strand DNA (dsDNA), anti-Sm, and anti-chromatin levels were determined by enzyme-linked immunosorbent assay, as previously described.[24] Some results are reported in equivalent dilution factors (EDF) of standardized reference MRL/lpr sera, as previously defined by the formula: equivalent dilution factors = (dilution of standard reference sera which gives the equivalent optical density of the test serum) $\times$ 10^{6}.[25]

Assessment of Nephritis

Proteinuria was estimated by Uristix reagent strips (Miles Laboratories, Elkhart, IN) at monthly intervals starting at 3 months of age. At the time of sacrifice (5 months), one kidney was fixed in 10% buffered formalin and processed for paraffin embedding and sectioning, followed by hematoxylin and eosin (H&E) staining and histological evaluation. The other kidney was frozen in OCT medium and processed for immunofluorescence staining of IgG and C3 with fluorescein isothiocyanate-conjugated goat anti-mouse IgG and C3 F(ab′)2 fragments (used at 1:75 for anti-IgG and 1:500 for anti-C3; Fisher/ICN, Durham, NC). The presence and severity of nephritis was determined as previously described.[26,27] A blinded observer (MP Madaio) evaluated and scored independently the severity of glomerular, interstitial, and vascular lesions by light microscopy. Similarly, the presence of glomerular, tubular basement membrane, and vascular deposits of IgG and C3 were graded by immunofluorescence microscopy, as described.[27,28] Multiple sections at a minimum of two different levels were observed. Each section typically involved evaluation of >25 glomeruli, >10 blood

vessels, and the interstitium contained within two to three longitudinal sections of kidney.

Assessment of Dermatitis

Mice were inspected monthly for the development of dermatitis, and the age at which open skin lesions developed was recorded. At the time of sacrifice (5 months), skin samples from the dorsal neck area (lesional and perilesional when present) were fixed in 10% buffered formalin and processed for paraffin embedding and sectioning, followed by H&E staining and histological evaluation. Additional skin samples were frozen in OCT and processed for immunofluorescence staining. Skin samples from younger mice (2.5 months old) with early stages of lesion development were also harvested and processed in a second experiment by following the same procedures. Skin cryosections were made at 5 μm, fixed in 95% ethanol or acetone, and processed for direct or indirect immunostaining, as described.[29] Skin sections were stained for the deposition of mouse C3 and IgG using fluorescein isothiocyanate-conjugated goat anti-mouse C3 or IgG antibodies (F(ab′)2 fragment for C3 from Fisher/ICN, used at 1:500 dilution; and whole molecule for IgG from Santa Cruz Biotechnology, Santa Cruz, CA, used at 10 μg/ml). In addition, skin sections were also stained for IgM deposition and for the presence of T lymphocytes using fluorescein isothiocyanate-conjugated goat anti-mouse IgM (used at 5 μg/ml; Sigma Chemical Co., St. Louis, MO) or rat anti-mouse CD4 and CD8 monoclonal antibodies (both used at 10 μg/ml; PharMingen, San Diego, CA). T cells were detected by using the horseradish peroxidase-avidin system with a kit from Vector Laboratories, Burlingame, CA. Staining of DAF in the skin was performed by using a polyclonal rabbit anti-mouse DAF antibody (antibody no. 1, used at 1:2000; see below),[30] followed by a fluorescein isothiocyanate-conjugated rat anti-rabbit IgG (1:200, PharMingen).

Analysis of Secondary Lymphoid Organs

The spleen and all identifiable lymph nodes were collected at the time of sacrifice, and wet weights and cell counts were determined. Cell suspensions were processed for immunofluorescence staining and flow cytometry analysis using FACScan with CellQuest data acquisition software (Becton Dickinson Immunocytometry Systems, San Jose, CA), as described.[24] Lymphocyte activation markers were analyzed by using monoclonal antibodies from PharMingen [CD23 (FcϵR), clone B3B4; CD44, clone IM7; CD69 (VEA), clone H1.2F3; CD86 (B7.2), clone GL1].

Northern and Western Blot Analyses of DAF and Crry Expression in the Skin and Kidney

Total RNAs were isolated from MRL/lpr or MRL/lpr-DAFKO mouse skin, kidney, lung, and testis using the Trizol reagent (Life Technologies, Springs, NY). Mouse lung and testis are known to express high levels of DAF and Crry[18–20,31] and were used as positive controls. Northern blot analysis was performed as previously described[19,23] using a GPI-DAF cDNA probe (nucleotides 1 to 1465,[20] hybridizes with both GPI-DAF and TM-DAF cDNAs), or a TM-DAF-specific probe (nucleotides 1134 to 1332[20]) or a Crry cDNA probe (nucleotides 608 to 1084[32]). For Western blot analysis, total proteins from different tissues of MRL/lpr and MRL/lpr-DAFKO mice were homogenized at 4°C in 10 vol of 50 mmol/L Tris buffer, pH 7.2, containing 1 mmol/L ethylenediaminetetraacetic acid, 1% Nonidet P-40, and a cocktail of protease inhibitors (Sigma Chemical Co.). Nuclei and cytoplasmic debris were then pelleted at 14,000 rpm using a microcentrifuge for 10 minutes at 4°C. Proteins from the resultant supernatant were separated on 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels (150 μg/lane) under nonreducing conditions. In some experiments, total protein extracts from vector- or mouse GPI-DAF cDNA-transfected human embryonic kidney 293 (HEK) cells[5] were run as control lanes. Western blot to detect DAF was performed using two independently generated polyclonal rabbit anti-mouse DAF antibodies. Antibody no. 1 was generated using purified murine erythrocyte DAF[30] and was kindly provided by Dr. Noriko Okada (Nagoya City University, Nagoya City, Japan). Antibody no. 2 was generated using a recombinant mouse DAF-Ig fusion protein and was kindly provided by Dr. Michael Holers (University of Colorado Health Sciences Center, Denver, CO). Western blot to detect Crry was performed using 1F2, a rat anti-mouse Crry monoclonal antibody from PharMingen. Signal detection was performed using the ECL Western blotting detection system from Amersham.

Results

To address the role of DAF in systemic autoimmune disease development, we crossed a DAF gene KO mouse previously generated in our laboratory[23] with the MRL/lprIgh^b mouse.[16,24] After four generations of backcrossing, a preliminary intercross experiment was performed, and DAFWT, heterozygous, and KO MRL/lprIgh^b mice were obtained as littermates. For this preliminary study, the WT and heterozygous mice were grouped together (n = 11). Comparison of these mice and a group of DAFKO littermates (n = 7) showed elevated serum IgG and anti-chromatin autoantibody titers in the KO mice at 3.5 and 5.5 months of age (data not shown). There were also signs that the group of DAFKO mice had enlarged spleens and lymph nodes and appeared to be more susceptible to skin inflammation and spontaneous ulcerative dermatitis development. Backcrossing to the MRL/lprIgh^b background was continued until the ninth generation, and DAF heterozygous mice were similarly intercrossed. Initial screening of the progeny identified 16 MRL/lprIgh^b DAFWT (DAFWT, +/+; 10 females and 6 males) and 11 MRL/lpr DAFKO mice (DAFKO, 7 females and 4 males). These mice were studied in detail for the development of systemic autoimmune disease until they

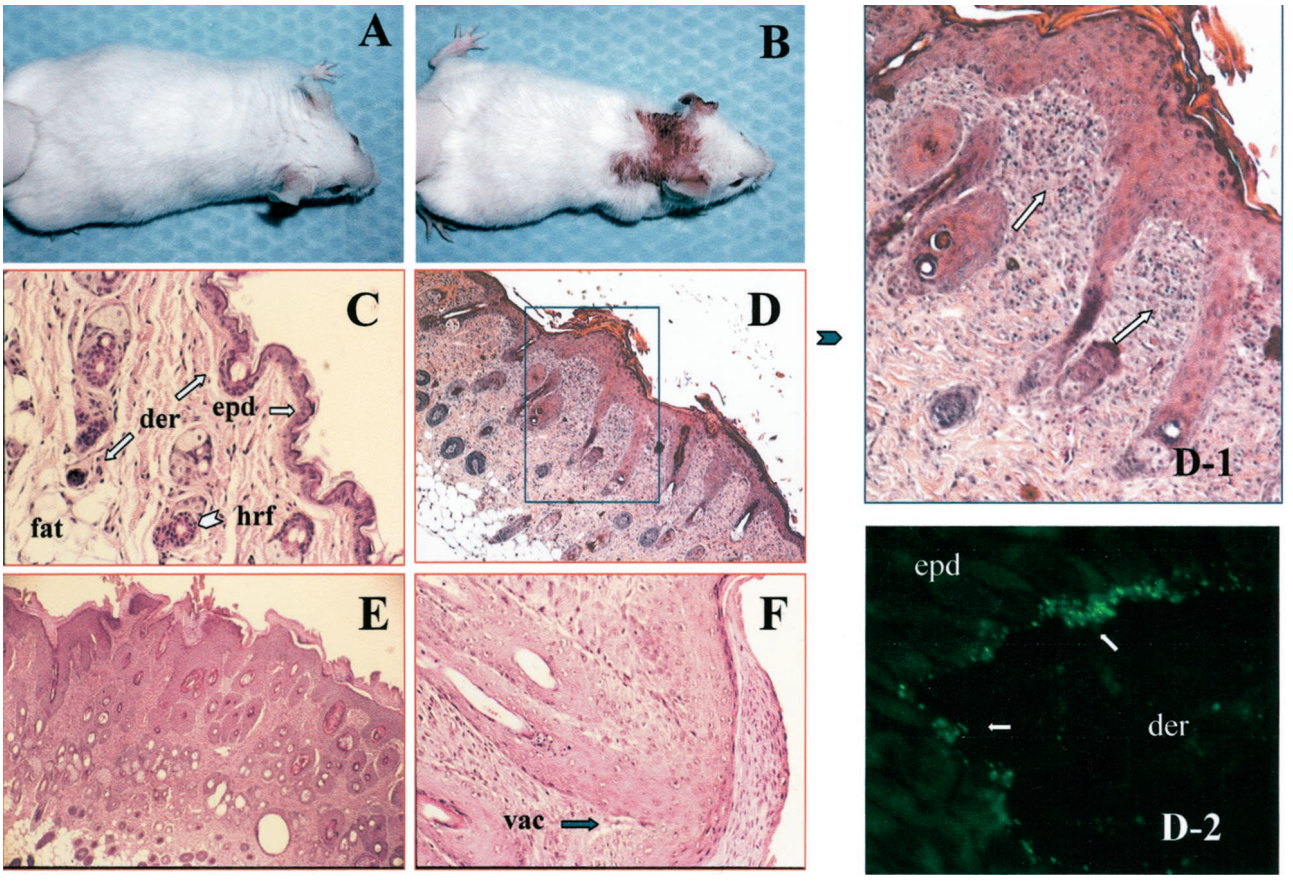

Figure 1. MRL/lpr DAFKO mice (**B**, 5 months old) developed aggravated dermatitis. None of the MRL/lpr DAFWT mice developed visible skin lesions at 5 months of age (**A**). Histology of normal mouse skin, showing clearly defined epidermal (epd), dermal (der), and fat layers and hair follicles (hrf), is presented in **C** (5-month-old WT mouse). **D** and **D-1** show the histology of an early skin lesion in a MRL/lpr-DAFKO mouse (2.5 months old). Skin thickening, mostly because of acanthosis of the epidermis, and inflammatory infiltrate (**arrows** in **D-1**) at the dermal/epidermal junction are evident. Focal C3 deposition along the dermal/epidermal junction was also observed in the early lesions (**arrows** in **D-2**). C3 staining was not observed in MRL/lpr DAFWT mouse skin or in the skin of a C3 KO mouse[45] used as a negative control (data not shown). **E** and **F** show pictures of an advanced skin lesion in a MRL/lpr DAFKO mouse at 5 months of age. Severe acanthosis and fibrosis but less inflammatory infiltrates are present. It is of interest that although signs of vacuolization (vac) are present at the dermal/epidermal junction in some sections (**arrow** in **F**), vacuolization did not appear to be a prominent feature in the fully developed lesions. Original magnifications: ×200 (**C**, **D-1**, **D-2**, **F**); ×100 (**D**, **E**).

were 5 months old. Data presented below were from the study of these fully backcrossed mice.

DAFKO MRL/lprlgh^b Mice Developed Accelerated and Aggravated Dermatitis

A striking phenotype that emerged from monitoring the cohort was the propensity of the DAFKO mice to develop accelerated and aggravated dermatitis. By 5 months of age, six of the seven female and one of the four male DAFKO mice had developed ulcerative dorsal skin lesions (Figure 1B). One of the female DAFKO mice was observed to have developed a full-thickness, ulcerated dorsal skin lesion at 3 months of age (similar to that shown in Figure 1B). In contrast, at the time of sacrifice, none of the 16 MRL/lpr DAFWT mice (10 females and 6 males) developed comparable skin problems (Figure 1A). Although spontaneous dermatitis is a well-described manifestation of MRL/lpr phenotype,[16] severe skin lesions usually develop sporadically and mostly at a more advanced age. Thus, the severity and high penetrance of dermatitis that we observed in the female DAFKO cohort was quite remarkable.

Histological analysis of the lesions in 5-month-old mice revealed skin thickening, mostly because of acanthosis of the epidermis (Figure 1, E and F). A few scattered inflammatory cells were noted, as well as some dermal fibrosis. Unlike some forms of human SLE dermatitis or the previously described MRL/lpr skin disease (spontaneous or ultraviolet-induced),[29] there was less vacuolization and no lichenoid inflammatory infiltrate at the dermal/epidermal junction (Figure 1, E and F). Immunostaining detected no IgG, IgM, or C3 deposition in the lesional areas. Weak C3 staining, some along the dermal/epidermal junction as well as at other levels of the epidermis, were observed in the perilesional skin tissues although, once again, the pattern of C3 deposition was less prevalent and less localized to the dermal-epidermal junction, distinct from that typically seen in the UV-induced murine dermatitis or human lupus dermatitis.[29] Immunostaining revealed the presence of some $CD4^+$ and CD8+ T cells in the lesional areas of 5-month-old mice (data not

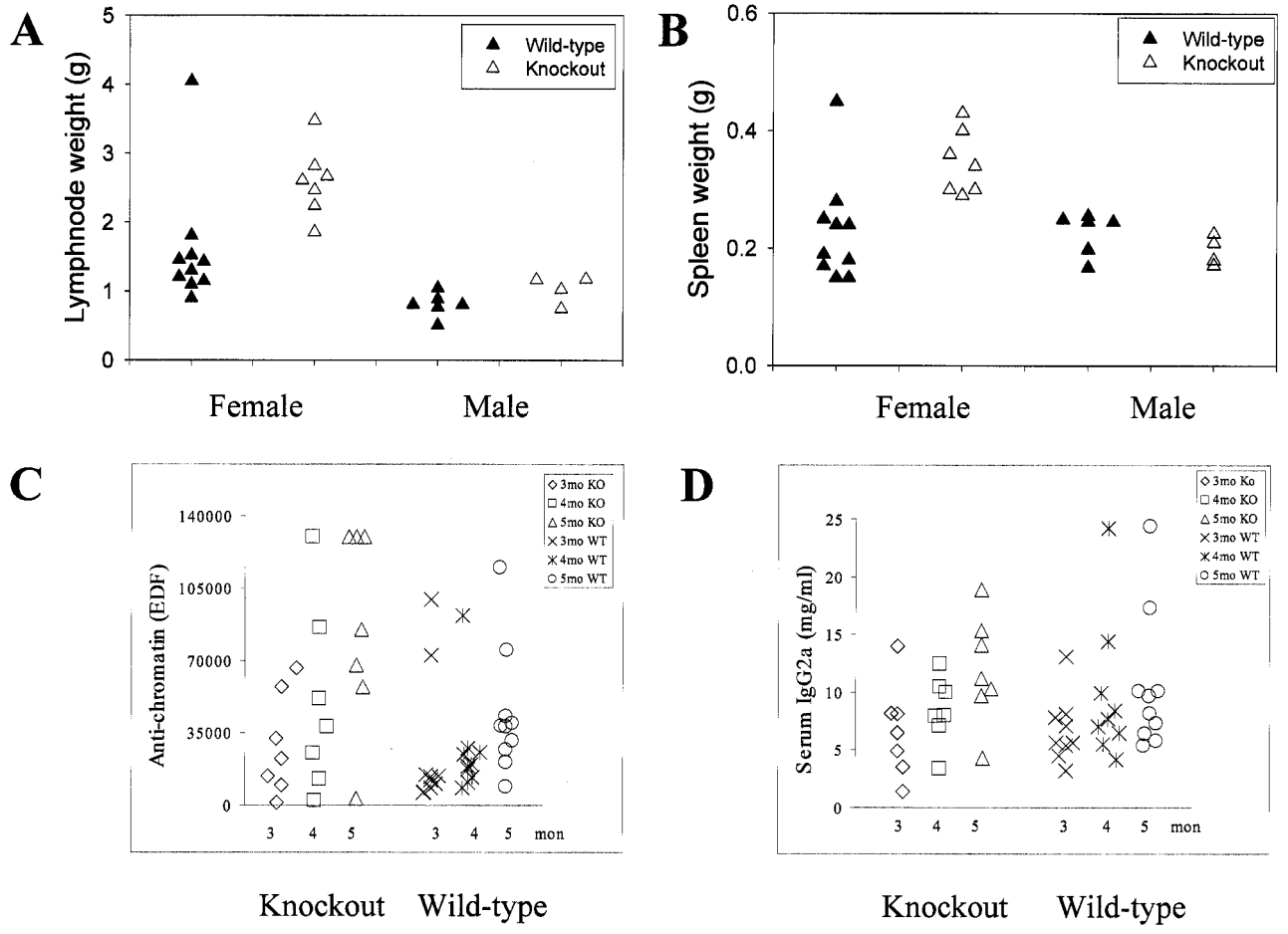

Figure 2. MRL/lpr DAFKO female mice had significantly enlarged lymph nodes (**A**, $P < 0.05$, Student's *t*-test) and spleens (**B**, $P < 0.01$, Student's *t*-test). It is of interest that one WT mouse also had very large lymph nodes and spleen, suggesting influence of other background genes. The fact that this mouse did not develop skin disease suggests dissociation between the lymphadenopathy and dermatitis phenotypes. **C** shows that serum anti-chromatin autoantibody titers in MRL/lpr DAFKO female mice were significantly increased at 5 months ($P < 0.05$, Student's *t*-test,). Serum IgG2a titers at 4 and 5 months (**D**), as well as anti-chromatin titers at 3 and 4 months (**C**) also appeared to be higher in the DAFKO mice, although they did not reach statistical significance because of the small sample sizes of this cohort.

shown). To gain some insight into the pathogenesis of the observed dermatitis, histological analysis of skin lesions at an earlier stage of development (in 2.5-month-old DAFKO mice) was performed in a separate experiment. As shown in Figure 1; D, D-1, and D-2, there was abundant inflammatory infiltrate as well as focal C3 deposition along the dermal-epidermal junction in these early lesions. The exact composition and time course of the inflammatory infiltrate in these early lesions remain to be completely characterized.

DAF Deficiency Exacerbated Lymphadenopathy and Splenomegaly in Female MRL/lprlgh^b Mice

At the time of sacrifice, DAFKO MRL/lprlgh^b mice had enlarged lymph nodes and spleens. This was particularly significant in the female mice. The wet weights of lymph nodes and spleens of the female DAF-deficient MRL/lprlgh^b mice were approximately twice that of the MRL/lprlgh^b DAFWT littermates (Figure 2, A and B). The increases in lymph node and spleen weights were because of a generalized increase in total cell numbers rather than selective expansion of specific lymphocyte populations. FACS analysis revealed no significant difference in the percentage of B cells, CD4$^+$, CD8+, or CD4/CD8-double-negative T cells, or in the expression of B- or T-cell surface activation markers (CD23, CD44, CD69, CD86) in these lymphoid organs (data not shown).

DAF Deficiency Increased Anti-Chromatin Autoantibody Production in Female MRL/lprlgh^b Mice

Although we detected elevated levels of serum total IgG and anti-chromatin autoantibodies in preliminarily backcrossed (fourth generation) DAFKO MRL/lprlgh^b mice, the difference in serum total IgG was no longer observed in the fully backcrossed DAFKO mice. Anti-chromatin autoantibody titers at 5 months were, however, still significantly elevated in the fully backcrossed female MRL/lprlghb DAFKO mice compared to DAFWT littermates ($P < 0.05$, Student's *t*-test; Figure 2C). Titers of anti-chromatin at 3 and 4 months were also higher in the

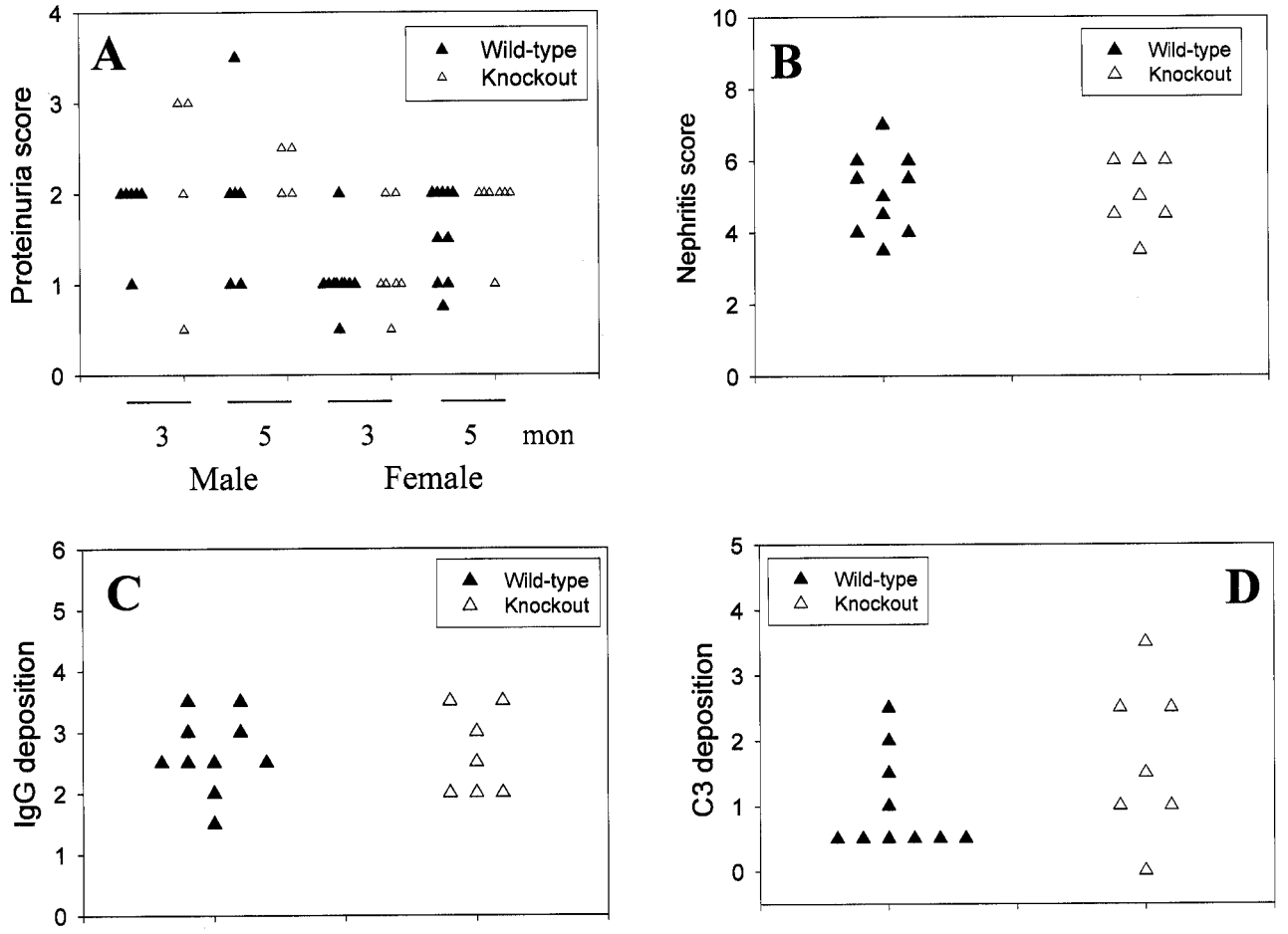

Figure 3. MRL/lpr DAFKO mice developed comparable nephritis as MRL/lpr DAFWT mice. Scatter plot of proteinuria scores of WT and DAFKO male and female mice at 3 and 5 months are shown in **A**. Cumulative nephritis scores (**B**), glomerular IgG (**C**), and C3 (**D**) deposition of the female cohort are shown in **B** to **D**.

DAFKO females but the increase did not reach statistical significance. Likewise, IgG2a levels, the dominant IgG isotype in MRL/lpr mice, appeared to be elevated at 4 and 5 months in female DAFKO mice, although this again was not statistically significant (Figure 2D). The failure to achieve a statistical significance in these values may reflect the relatively small sample size of this cohort of mice. No differences in the titers of serum IgM, anti-ssDNA, anti-dsDNA, anti-Sm, or rheumatoid factor (RF) (anti-IgG1) were observed (data not shown).

DAF Deficiency Did Not Significantly Impact the Development of Nephritis in MRL/lprlgh^b Mice

Although the average score of proteinuria (semiquantitative) appeared to be higher in the DAFKO mice, nephritis was not significantly greater in these mice, as compared with DAFWT mice (Figure 3A). This conclusion was confirmed by subsequent histopathology evaluations. The cumulative nephritis score was not significantly different between the two groups of mice (Figure 3B). There was also no significant difference in glomerular IgG deposition (Figure 3C). There was a trend toward increased glomerular C3 deposition (6 of 7 DAFKO and 4 of 10 DAFWT mice had a C3 deposition score of 1 or greater, Figure 3D), but the difference was not statistically significant ($P = 0.13$, two-tailed Fisher's exact test). Thus, DAF deficiency did not significantly impact the development of nephritis in these mice.

DAF and Crry Are Differentially Expressed in the Mouse Skin and Kidney

In light of the high prevalence of dermatitis observed in the DAFKO mice, we investigated the relative expression of DAF and a second major rodent C3 convertase inhibitor, complement receptor 1-related gene y (Crry), in the mouse skin and kidney. DAF was found to be expressed prominently in the skin of normal MRL/lpr mice as revealed by Northern blot analysis (Figure 4A). Its expression in the skin was comparable to that in the mouse lung, a tissue that is known to express a high level of DAF.[18,19] On the other hand, DAF is expressed at a very low level in the MRL/lpr mouse kidney (Figure 4A). As expected, no DAF mRNA signal was detected in the DAFKO mouse tissues (skin, kidney, or lung; Figure 4A). When the expression of Crry, a membrane C3 regulator with DAF and membrane cofactor protein activities that is uniquely ex-

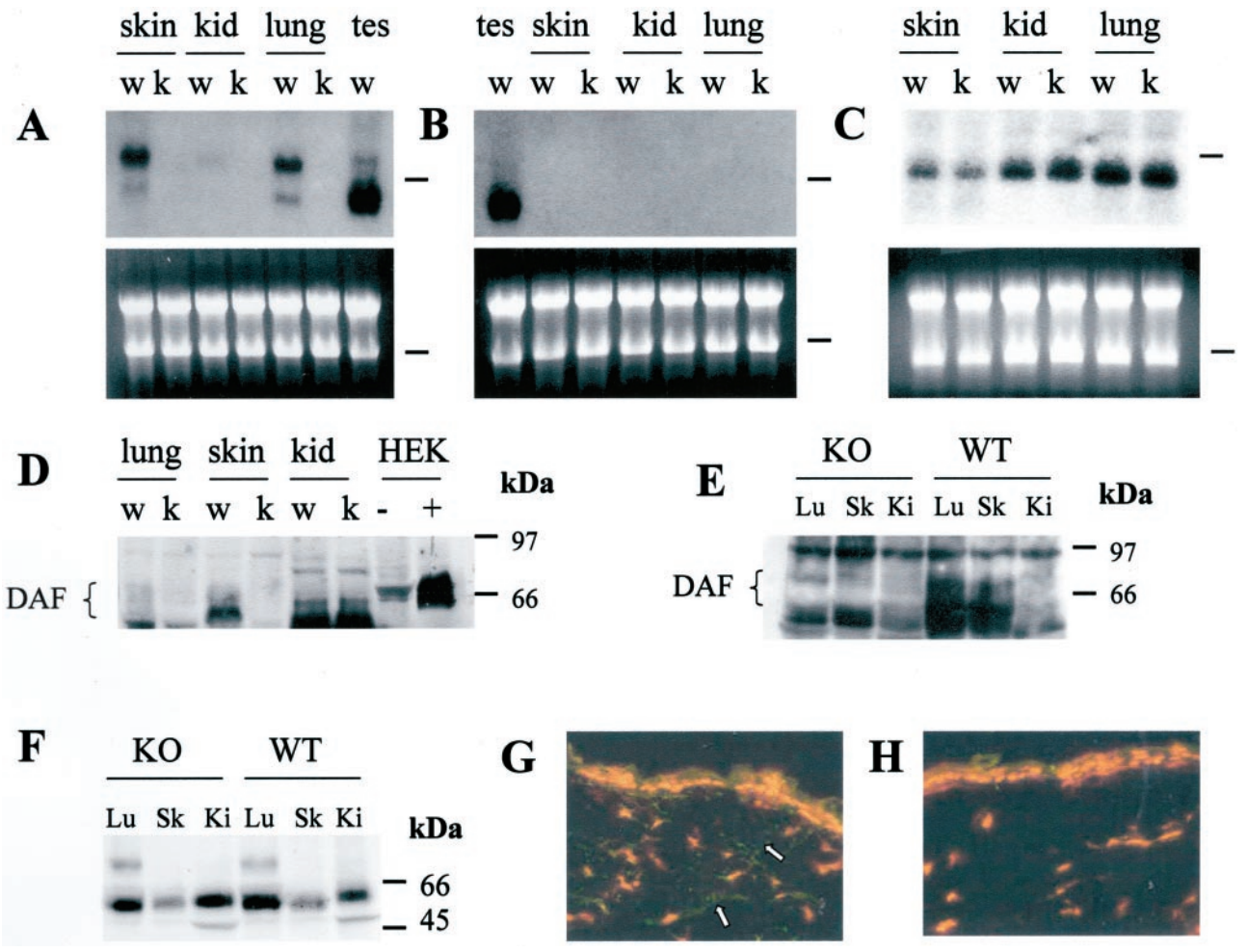

Figure 4. Northern blot (**A–C**) and Western blot (**D–F**) analysis showing differential expression of DAF and Crry in the mouse skin and kidney (kid, Ki). In **A–C**, RNA samples from WT (w) or DAFKO (k) MRL/lpr mice were separated and the blot was hybridized with either a GPI-DAF cDNA probe (which recognizes both GPI-DAF and TM-DAF cDNAs) (**A**), or a TM-DAF-specific cDNA probe (**B**), or a Crry cDNA probe (**C**). RNAs from the mouse lung and testis (tes) were used as positive controls. Two GPI-DAF mRNA species, with variable relative abundance in different tissues, are known to be transcribed.[18,19] The position of the 18S ribosomal RNA was indicated by a **horizontal bar** in **A–C**. In **D–F**, total proteins from the WT (w, WT) or DAFKO (k, KO) MRL/lpr mouse lung (Lu), skin (Sk), or kidney (kid, Ki), as well as proteins from vector-transfected (−) or mouse DAF cDNA-transfected (+) HEK cells, were separated on 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotted with anti-mouse DAF polyclonal antibody no. 1 (**D**) or antibody no. 2 (**E**) or a monoclonal antibody against mouse Crry (**F**). DAF protein expressed in HEK cells (lane marked with + in **D**) migrated as a broad 66-kd band, reflecting its glycoprotein nature.[1,46] The signal in the vector-transfected HEK cells (lane marked with − in **D**) may represent human DAF endogenous to HEK cells. Note the apparent difference between WT and KO mice in lung and skin, but not kidney DAF signals (**D**, **E**). Immunofluorescence analysis of skin (with anti-DAF polyclonal antibody no. 1) showed DAF to be primarily expressed in the elastic fibers (bright green, **arrows** in **G**). No specific staining was detected in the DAFKO mouse skin (**H**). Orange color in **G** and **H** represents counterstain with propidium iodide.

pressed in the mouse and rat,[31,33] was examined, a different pattern was observed. Crry was expressed abundantly in the MRL/lpr mouse kidney and lung but its expression in the mouse skin was comparatively low (Figure 4C). The differential expression of DAF and Crry in the MRL/lpr mouse skin and kidney, ie, DAF is relatively high in the skin and low in the kidney whereas Crry is low in the skin and high in the kidney, was confirmed by Western blot analysis (Figure 4, D to F). Notably, no expression of the TM-DAF (Daf-2) gene was detected in the WT or GPI-DAFKO MRL/lpr mouse skin, kidney, or lung (Figure 2B). Immunofluorescence staining showed DAF to be expressed primarily in the elastic fibers of the mouse skin (Figure 4, G and H). This is similar to the localization pattern of DAF in the human skin.[34]

Discussion

The results of this study provide evidence for a protective role for DAF in systemic autoimmune disease development. MRL/lprIgh^{b} DAFKO mice developed increased lymphoproliferation, anti-chromatin autoantibody production, and dermatitis. These phenotypes were more prominent in females, which is likely a reflection of the known gender bias in disease manifestation intrinsic to the MRL/lpr background.[16] There are some notable characteristics in the histopathology of dermatitis in older (5 months old) MRL/lprIgh^{b} DAFKO mice that were distinct from that previously described for MRL/lpr mice.[29] There was less vacuolization and no lichenoid inflammatory infiltrate at the dermal/epidermal junction in the diseased skins. On

the other hand, there was abundant inflammatory infiltrate at the dermal/epidermal junction in early-stage lesions of 2.5-month-old mice. It is also of interest that disease exacerbation by DAF deficiency is somewhat selective. Deletion of DAF affected some aspects (eg, dermatitis and lymphoproliferation) but not others (eg, nephritis). A similar effect has been reported in another congenic line, the β2-microglobulin-deficient MRL/lpr mouse[35] and MA Maldonado, unpublished observation). In these mice, all disease manifestations are suppressed except for the dermatitis, which is also aggravated.[35]

Exacerbation of autoimmune disease in DAFKO MRL/lpr mice is most likely a result of the deletion of a functional GPI-DAF gene, although the possibility cannot be entirely excluded that some 129J strain-derived background gene(s) linked to the DAF gene locus were responsible. As mentioned earlier, the two DAF genes are localized on mouse chromosome 1[18] and several lupus susceptibility genes have previously been mapped to this chromosome in the (NZB $\times$ NZW)F1 murine model of lupus.[36] This question may be addressed in the future by the generation of a MRL/lpr mouse congenic for the region of 129 mouse chromosome 1 that contains the DAF gene or by DAF transgenic rescue experiments in MRL/lpr-DAFKO mice. The beneficial role of DAF in MRL/lpr mice could involve both complement-dependent and -independent mechanism(s). DAF is best known as a regulator of C3 activation on the surface of autologous cells.[17] In the mouse, Crry is a second membrane-associated C3 inhibitor that overlaps with DAF both in its activity and tissue distribution[31,33] with one previously known exception in the embryos, where Crry but not DAF is expressed.[5,37] Interestingly, Crry KO mouse embryos were found to be susceptible to lethal maternal complement attack.[37] DAF and Crry may compensate each other's function, and in the absence or reduced expression of both inhibitors, complement-mediated tissue damage, particularly in an autoimmune disease setting, may occur. The accelerated dermatitis may therefore be explained by limited or differential expression of Crry in the mouse skin that is not up-regulated by the absence of DAF. Indeed, Northern and Western blot analysis revealed that DAF is abundantly expressed in the mouse skin whereas the expression of Crry in the mouse skin is relatively low compared with other tissues such as the kidney (Figure 4).

Exacerbation of disease in DAFKO MRL/lpr mice could be because of an effect of DAF deficiency on either the induction or effector phase of autoimmune disease development. Although IgG, IgM, and C3 deposition was not detected in fully developed open skin lesions at the time of sacrifice, increased complement-mediated skin damage may occur at earlier stages of lesion development. Indeed, focal C3 staining was observed at the dermal/epidermal junction in early stage lesions from younger (2.5 months old) MRL/lpr-DAFKO mice (Figure 1). The significant enlargement of spleens and lymph nodes in female DAFKO mice suggests that DAF may also play a role in the induction phase of autoimmunity. The lack of influence of DAF deficiency on MRL/lpr nephritis was notable. In a recent study of nephrotoxic serum nephritis using a low dose of anti-glomerular basement membrane antibody, we have demonstrated a protective role of DAF in the effector phase of glomerulonephritis in C57BL/6 mice.[38] The lack of effect of DAF deficiency on MRL/lpr mouse kidney disease may reflect low constitutive expression of DAF and adequate compensation by Crry in MRL/lpr mouse kidney (Figure 4). Alternatively, it may suggest that in this model of chronic autoimmune nephritis, complement-independent pathways are operative.[28] It is also possible that deficiency of DAF has affected additional antibody and T-cell-mediated events in lupus nephritis, which makes it difficult to discern a phenotypic difference.

DAF could affect the induction phase of autoimmunity in MRL/lpr mice via two potential mechanisms. Firstly, absence of DAF on antigen-presenting cells and other host cells (eg, in the skin) may result in enhanced complement opsonization and promote local inflammation. Such events could exacerbate the autoimmune response through complement receptor (CR; eg, CR1 and CR2) pathways or cytokine-mediated events. The adjuvant effect of complement fragment tagging to foreign antigens on the humoral response of host animals is well established.[39–41] One might argue that a similar effect of C3 tagging to autoantigens in the absence of membrane regulators of complement may exist and enhance cellular and humoral immune responses in mice predisposed to autoimmunity. C3 deposition on antigen-presenting cells also lowers the activation threshold of antigen-specific T cells.[42] These mechanisms may explain our observed increases in some autoantibody assays and exacerbated lymphoproliferation in DAFKO MRL/lpr mice. Secondly, interaction of DAF with CD97 may provide a complement-independent mechanism to regulate cellular and humoral immune reactions. In addition to functioning as a complement inhibitor, DAF is a ligand for the activation-associated lymphocyte antigen CD97.[2,3] CD97, a seven-span transmembrane protein with sequence homology to type II G-protein-coupled cell surface receptors, is potentially involved in lymphocyte/macrophage activation and cell signaling.[43,44] It is possible that DAF-CD97 interaction provides a negative regulatory mechanism for the autoimmune reactions in the MRL/lpr model. An uncoupling of normal T:B cell interactions because of DAF deficiency could also explain the increase in antibody levels and other aspects of increased autoimmunity. Ongoing experiments in our laboratory involving the crossbreeding of DAFKO MRL/lpr and C3 KO MRL/lpr mice and analysis of autoimmune diseases in their progeny should help to differentiate these two possibilities. Regardless of the molecular mechanisms, our finding of exacerbated autoimmune disease in DAFKO MRL/lpr mice suggests that dysfunction of DAF may also increase the risk for systemic autoimmune disease development in humans.

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